PfuTurbo® DNA Polymerase

INSTRUCTION MANUAL

Catalog #600250 , #600252 , and #600254
Revision #010001d

STORAGE CONDITIONS

–20°C

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PfuTurbo® DNA Polymerase

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PfuTurbo® DNA Polymerase

INTRODUCTION

PfuTurbo® DNA polymerase* is an enhanced version of *Pfu* DNA polymerase* for high-fidelity PCR.[1] *PfuTurbo* DNA polymerase is a blend of cloned *Pfu* DNA polymerase and a novel thermostable factor** that enhances PCR product yields without altering DNA replication fidelity. *PfuTurbo* DNA polymerase can be used to amplify complex genomic DNA targets up to10kb and vector targets up to 15kb in length. In general *PfuTurbo* DNA polymerase amplifies complex targets in higher yield than *Taq* DNA polymerase or other commercially-available proofreading PCR enzymes.[1] The error rate of *PfuTurbo* DNA polymerase is equal to that of *Pfu* DNA polymerase[1] and significantly lower than the error rates of other proofreading enzymes[2], DNA polymerase mixtures[2,3,4], and *Taq* DNA polymerase[2]. Finally, the enhanced performance of *PfuTurbo* DNA polymerase allows the use of shorter extension times, fewer PCR cycles, and lower DNA template concentrations than are required for *Pfu* DNA polymerase. These features of *PfuTurbo* DNA polymerase (e.g. fidelity, sensitivity, and yield) make it ideally suited for high performance PCR applications.

MATERIALS PROVIDED

	Quantity		
Materials provided	**Catalog #600250**	**Catalog #600252**	**Catalog #600254**
PfuTurbo® DNA polymerase (2.5 U/ µl)	100 U[a]	500 U[b]	1000 U[c]
10× Cloned Pfu DNA polymerase reaction buffer[d]	1 ml	2 × 1 ml	4 × 1 ml

[a] Sufficient *PfuTurbo* DNA polymerase is provided for up to 40 100-µl reactions.
[b] Sufficient *PfuTurbo* DNA polymerase is provided for up to 200 100-µl reactions.
[c] Sufficient *PfuTurbo* DNA polymerase is provided for up to 400 100-µl reactions.
[d] See *Preparation of Media and Reagents.*

* U.S. Patent No. 5,545,552 and patents pending.
** Patents pending.
† See *Endnotes*.

Revision #010001d

CRITICAL OPTIMIZATION PARAMETERS FOR *PfuTURBO* DNA POLYMERASE-BASED PCR

Pfu DNA polymerase exhibits the lowest error rate of any thermostable DNA polymerase analyzed (Table I),[2,5–7] making *PfuTurbo* and *Pfu* DNA polymerase the ideal choice for high-fidelity PCR amplification. All PCR amplification reactions, whether performed using *Taq*, *Pfu*, or *PfuTurbo* DNA polymerase, require optimization to achieve the highest product yield and specificity. Critical optimization parameters for successful PCR using *PfuTurbo* DNA polymerase are outlined in the following sections and include the use of an extension time that is adequate for full-length DNA synthesis, sufficient enzyme concentration, optimization of the reaction buffer, adequate primer–template purity and concentration, and optimal primer design.

Extension Time

Extension time is one of the most critical parameters affecting the yield of PCR product obtained using *PfuTurbo* DNA polymerase. For optimal yield with minimal smearing using *PfuTurbo* DNA polymerase, use an extension time of 1.0 minute/kb for vector targets up to 10kb and genomic targets up to 6kb. When amplifying vector targets between 10 and 15kb or genomic targets between 6 and 10kb in length, use an extension time of 2.0 minutes/kb. In contrast, *Pfu* DNA polymerase-based PCR amplifications require a minimum extension time of 2.0 minutes/kb of amplified template to achieve similar target synthesis.[8]

Enzyme Concentration

The concentration of *PfuTurbo* DNA polymerase required for optimal PCR product yield and specificity depends on the individual target system to be amplified. Most amplifications are successful with 2.5–5.0 U of enzyme/100-µl reaction for vector targets up to 15kb and genomic targets up to 10kb.

TABLE I

Fidelity Comparison of Thermostable DNA Polymerases Using a *lacI*OZα-Based Fidelity Assay[a]

Thermostable DNA polymerase	Error rate[b]	Percentage (%) of mutated PCR products[c]
PfuTurbo DNA polymerase	1.3×10^{-6}	2.6
Pfu DNA polymerase	1.3×10^{-6}	2.6
Taq DNA polymerase	8.0×10^{-6}	16.0
Vent$_R$® DNA polymerase	2.8×10^{-6}	5.6
Deep Vent$_R$® DNA polymerase	2.7×10^{-6}	5.4
Tfl DNA polymerase	8.3×10^{-6}	16.6
Tbr DNA polymerase	9.5×10^{-6}	19.0
UlTma™ DNA polymerase	55.3×10^{-6}	110.6[d]

[a] Fidelity is measured using a PCR-based forward mutation assay based on the *lacI* target gene.[2]

[b] The error rate equals mutation frequency per base pair per duplication.

[c] The percentage of mutated PCR products after amplification of a 1-kb target sequence for 20 effective cycles (2^{20}- or 10^6-fold amplification).

[d] Some PCR products will exhibit more than one error.

Reaction Buffer

In *PfuTurbo* DNA polymerase-based PCR, the reaction buffer has been formulated for optimal PCR yield and fidelity.[2] Stratagene strongly recommends use of the reaction buffer provided with *PfuTurbo* DNA polymerase in standard PCR amplification reactions. If alterations in these buffers are made, significant increases in the error rate of *Pfu* DNA polymerase can be avoided by maintaining the Mg^{2+} concentration above 1.5 mM, the total dNTP concentration at or below 1 mM, and the pH of Tris-based buffers above pH 8.0 when measured at 25°C.[2] Deoxynucleoside triphosphate (dNTP) concentrations of 100–250 µM each dNTP generally result in the optimal balance of product yield (greatest at high dNTP concentrations) versus specificity and fidelity (highest at low dNTP concentration).[9] If additional buffer optimization is desired for a specific primer–template combination, the Opti-Prime™ PCR optimization kit[††] can be used. The Opti-Prime PCR optimization kit contains a variety of useful buffers, adjuncts, and cosolvents specifically selected for use in PCR and is configured in a testing matrix that simplifies optimization of reaction conditions.[10]

Primer–Template Purity and Concentration

The most successful PCR results are achieved when the amplification reaction is performed using purified primers and templates that are essentially free of extraneous salts. Gel-purified primers, generally >18 nucleotides in length, are strongly recommended for use in *PfuTurbo* DNA polymerase-based PCR.

Additionally, an adequate concentration of primers and template should be used to ensure a good yield of the desired PCR products. When DNA of known concentration is available, amounts of 50–1000 ng of DNA template/100-µl reaction are typically used for amplifications of single-copy chromosomal targets. The amplification of a single-copy target from complex genomic DNA is generally more difficult than amplification of a fragment from a plasmid or phage. Less DNA template can be used for amplification of lambda (1–30 ng) or plasmid (10–100 pg) PCR targets or for amplification of multicopy chromosomal genes (10–100 ng).[9] The mutation frequency can be reduced by limiting the number of PCR cycles; however, a corresponding increase in DNA template concentration is required in order to achieve comparable yields of PCR product.

Stratagene suggests using primers at a final concentration of 0.1–0.5 µM, which is equivalent to ~100–250 ng of an 18- to 25-mer oligonucleotide primer in a 100-µl reaction volume. Relatively high concentration of primers is typically required due to the fact that proofreading DNA polymerases such as *Pfu* DNA polymerase exhibit 3′- to 5′-exonuclease activity, which may contribute to a certain level of primer degradation. The use of primers with a phosphorothioate bond at the 3′-terminal internucleotide linkage reportedly minimizes primer degradation.[11]

[††] See *Endnotes.*

Primer Design

Primer pairs that exhibit similar melting temperatures and are completely complementary to the template are recommended. Depending on the primer design and the desired specificity of the PCR amplification reaction, melting temperatures between 55° and 80°C generally yield the best results.[9] The following formula[12] is commonly used for estimating the melting temperature (T_m) of the primers:

$$T_m(^\circ C) \cong 2(N_A + N_T) + 4(N_G + N_C)$$

where N equals the number of primer adenine (A), thymidine (T), guanidine (G), or cytosine (C) bases. Several other articles present additional equations for estimating the melting temperature of primers.[13,14] Finally, care must be taken when using degenerate primers. Degenerate primers should be designed with the least degeneracy at the 3′ end. Optimization of degenerate primer concentration is necessary.

Additional Optimization Parameters for *PfuTurbo* DNA Polymerase-Based PCR

PCR Cycling Parameters

Standard PCR amplification reactions typically require 25–30 cycles to obtain a high yield of PCR product. Because high fidelity is a concern for certain PCR applications such as expression cloning, Stratagene suggests using a minimum number of cycles for *PfuTurbo* DNA polymerase-based PCR to ensure the lowest number of errors. Thermal cycling parameters should be chosen carefully to ensure (1) the shortest denaturation times to avoid enzyme inactivation and/or template damage, (2) adequate extension times to achieve full-length target synthesis, and (3) the use of annealing temperatures near the primer melting temperature to improve yield of the desired PCR product.

When performing PCR on a new target system, Stratagene suggests using an annealing temperature 5°C below the lowest primer melting temperature.

Note *Stratagene's RoboCycler® Gradient 40 temperature cycler††† and RoboCycler® Gradient 96 temperature cycler††† quickly optimize PCR annealing temperature by testing up to 12 different temperatures in one experiment.*

For best results, PCR primers should be designed with similar melting temperatures ranging from 55° to 80°C. The use of primers with melting temperatures within this range reduces false priming and ensures complete denaturation of unextended primers at 94–95°C (see also *Primer–Template Purity and Concentration* and *Primer Design*).

See Table III for optimized PCR cycling parameters using Stratagene's RoboCycler 40. Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers. Therefore, each manufacturer's recommendations for optimal cycling parameters should be consulted.

††† See *Endnotes*.

Order of Addition of Reaction Mixture Components

Because *PfuTurbo* DNA polymerase exhibits 3′- to 5′-exonuclease activity that enables the polymerase to proofread nucleotide misincorporation errors, it is critical that *PfuTurbo* DNA polymerase is the last component added to the PCR mixture (i.e., **after** the dNTPs). In the absence of dNTPs, the 3′- to 5′-exonuclease activity of proofreading DNA polymerases may degrade primers. When primers and nucleotides are present in the reaction mixture at recommended levels (i.e., primer concentrations of 0.1–0.5 µM and nucleotide concentrations of 100–250 µM), primer degradation is minimal.

Deoxynucleoside Triphosphates

For *PfuTurbo* DNA polymerase-based PCR, Stratagene recommends a dNTP concentration range of 100–250 µM each dNTP (0.4–1.0 mM total). Deoxynucleoside triphosphate concentrations of 100–250 µM each dNTP generally result in the optimal balance of product yield (greatest at high dNTP concentrations) versus specificity and fidelity (highest at low dNTP concentration).[2,9] The use of a balanced pool of dNTPs (equimolar amounts of each dNTP) ensures the lowest rate of misincorporation errors.

Salt Concentrations

Magnesium Ion Concentration

Magnesium ion concentration affects primer annealing and template denaturation, as well as enzyme activity and fidelity. Generally, excess Mg^{2+} concentration results in accumulation of nonspecific amplification products, whereas insufficient Mg^{2+} concentration results in reduced yield of the desired PCR product.[15] PCR amplification reactions should contain *free* Mg^{2+} in excess of the total dNTP concentration (i.e., an optimal *free* Mg^{2+} concentration between 0.5 and 2.5 mM).[9] For *PfuTurbo* DNA polymerase-based PCR, fidelity is optimal when the *total* Mg^{2+} concentration is ~2 mM in a standard reaction mixture. This *total* Mg^{2+} concentration is present in the final 1× dilution of the cloned *Pfu* DNA polymerase 10× reaction buffer. The fidelity of *PfuTurbo* DNA polymerase drops significantly at a *total* Mg^{2+} concentration ≤1 mM in the presence of 200 µM each dNTP.

Note that the presence of ethylenediaminetetraacetic acid (EDTA) or other metal chelating agents lowers the effective concentration of Mg^{2+} as do excessive levels of dNTPs. The concentration of metal chelators and total dNTPs should be taken into account when determining the final Mg^{2+} concentration required for PCR.

Potassium Chloride Concentration

Potassium chloride or other salts increases the ionic strength of the reaction mixture, which alters the denaturing and annealing temperatures of DNA, as well as enzyme activity. Varying the KCl concentration at a given annealing temperature may improve the specificity and yield of the desired PCR product.

Adjuncts and Cosolvents

The adjuncts or cosolvents listed in the following table, which are available in the Opti-Prime PCR optimization kit, may be advantageous with respect to yield when used in the PCR buffer. Fidelity may or may not be affected by the presence of these adjuncts or cosolvents.

Adjunct or cosolvent	Optimal PCR final concentration
Bovine serum albumin (BSA)	10–100 µg/ml
Formamide	1.25–10%
Dimethylsulfoxide (DMSO)	1–10%
Glycerol	5–20%
Ammonium sulfate [$(NH_4)_2SO_4$]	15–30 mM
Perfect Match® PCR enhancer[††]	1 U/100-µl reaction (genomic DNA template)
	0.01–1 U/100-µl reaction (plasmid DNA template)

[††]See *Endnotes*.

Bovine Serum Albumin

Bovine serum albumin is a nonspecific enzyme stabilizer that also binds certain PCR inhibitors.[16]

Formamide

Formamide facilitates certain primer–template annealing reactions and also lowers the denaturing temperature of melt-resistant DNA.[17]

Dimethylsulfoxide and Glycerol

Cosolvents, such as DMSO and glycerol, improve the denaturation of GC-rich DNA and help overcome the difficulties of polymerase extension through secondary structures. Studies indicate that the presence of 1–10% DMSO in PCR may be essential for the amplification of the retinoblastoma gene[18] and may also enhance amplification of *Herpes simplex* virus (HSV) sequences.[19] Glycerol is known to improve the yield of amplification products and also serves as an enzyme stabilizer.[19]

Ammonium Sulfate

Ammonium sulfate increases the ionic strength of the reaction mixture, which alters the denaturing and annealing temperatures of DNA, as well as enzyme activity.

Perfect Match PCR Enhancer

Perfect Match PCR enhancer improves the specificity of PCR products. This adjunct performs these functions by destabilizing mismatched primer–template complexes and helps to remove secondary structures that could impede normal extension.[20]

APPLICATION NOTES

Thermostability

Pfu DNA polymerase is a highly thermostable enzyme, retaining 94–99% of its polymerase activity after 1 hour at 95°C. Unlike *Taq* DNA polymerase, denaturing temperatures up to 98°C can be used successfully with *PfuTurbo* DNA polymerase to amplify GC-rich regions.[21,22]

Inherent "Hot Start" Properties

Pfu DNA polymerase exhibits optimal polymerase activity at ≥75°C and only 2–8% activity between 40–50°C. *Taq* DNA polymerase, however, exhibits optimal polymerase activity between 60–70°C and 27–70% activity between 40–50°C. The minimal activity of *PfuTurbo* DNA polymerase at lower temperatures should result in fewer mispaired primer-extension reactions than occur with *Taq* DNA polymerase during the lower temperatures encountered during PCR cycling (e.g., primer annealing). Consequently, hot start techniques, which are commonly used with *Taq* DNA polymerase to improve product yield and specificity, generally are not required for PCR amplifications with *Pfu* DNA polymerases.[23]

Terminal Transferase Activity

Studies demonstrate that thermostable DNA polymerases, with the exception of *Pfu* DNA polymerase, exhibit terminal deoxynucleotidyltransferase (TdT) activity, which is characterized by the addition of nontemplate-directed nucleotide(s) at the 3′ end of PCR-generated fragments.[24,25] *Pfu* DNA polymerase is devoid of TdT activity and generates blunt-ended PCR products exclusively. Therefore, *PfuTurbo* and *Pfu* DNA polymerases are the enzymes of choice for use with the PCR-Script™ Amp SK(+) cloning kit[26,††] and the PCR-Script™ Cam SK(+) cloning kit.[27,††] Alternatively, *PfuTurbo* or *Pfu* DNA polymerase can be used to remove 3′ overhangs (polishing) or to fill-in 5′ overhangs with greater efficiencies than either Klenow polymerase or T4 DNA polymerase.[29,30]

Reverse Transcriptase Activity

PfuTurbo DNA polymerase lacks detectable reverse transcriptase activity.

[††] See *Endnotes.*

PCR PROTOCOL USING *PfuTURBO* DNA POLYMERASE

1. Prepare a reaction mixture for the appropriate number of samples to be amplified. Add the components *in order* while mixing gently. Table II provides an example of a reaction mixture for the amplification of a typical single-copy chromosomal target. The recipe listed in Table II is for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 100 µl.

   Note *The volumes of each component in the reaction mixture may also be decreased proportionally to a 50-µl final volume.*

2. Immediately before thermal cycling, aliquot 100 µl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes or standard 0.5-ml microcentrifuge tubes.

3. Overlay each reaction with ~50 µl of DNase-, RNase-, and protease-free mineral oil (Sigma, St. Louis, Missouri).

4. Perform PCR using optimized cycling conditions (see also *PCR Cycling Parameters*). Suggested cycling parameters for *PfuTurbo* DNA polymerase-based PCR using Stratagene's RoboCycler 40 temperature cycler are indicated in Table III.

5. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

TABLE II

Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification

Component	Amount per reaction
Distilled water (dH_2O)	81.2 µl
10× cloned *Pfu* DNA polymerase reaction buffer	10.0 µl
dNTPs (25 mM each dNTP)	0.8 µl
DNA template (100 ng/µl)	1.0 µl[a]
Primer #1 (100 ng/µl)	2.5 µl[b]
Primer #2 (100 ng/µl)	2.5 µl[b]
PfuTurbo DNA polymerase (2.5 U/µl)	1.0 µl (2.5 U)[c]
Total reaction volume	100 µl

[a] The amount of DNA template required varies depending on the type of DNA being amplified. Generally 50–1000 ng of genomic DNA template is recommended; however, less DNA template (typically 10 pg–100 ng) can be used for amplification of lambda or plasmid PCR targets or for amplification of multicopy chromosomal genes.

[b] Primer concentrations between 0.1 and 0.5 µM are recommended (generally 100–250 ng for typical 18- to 25-mer oligonucleotide primers in a 100-µl reaction volume).

[c] The amount of *PfuTurbo* DNA polymerase varies depending on the length of the template to be amplified. The standard range for vector targets up to 15 kb and genomic targets up to 10 kb in length is 1–2 µl (2.5–5.0 U).

TABLE III

Suggested Cycling Parameters for PCR Using *PfuTurbo* DNA Polymerase[a,b]

Segment	Number of cycles	Temperature	Duration
1[c]	1	94–98°C[d]	1 minute
2	25–30	94–98°C	1 minute
		Primer T_m – 5°C[e]	1 minute
		72°C	1 minute/kb of PCR target[f]
3	1	72°C	10 minutes

[a] Optimized for *PfuTurbo* DNA polymerase-based PCR using Stratagene's RoboCycler 40 temperature cycler.

[b] Thin-wall PCR tubes are highly recommended for use with Stratagene's thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance.

[c] Certain thermocyclers may require the removal of segment 1 from the cycling parameters listed to obtain the suggested cycling parameters for *PfuTurbo* DNA polymerase-based PCR. Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers; therefore, each manufacturer's recommendations for optimal cycling parameters should be consulted.

[d] Denaturing temperatures above 95°C are recommended only for GC-rich templates.

[e] The annealing temperature may be lowered further if necessary to obtain optimal results. Typically annealing temperatures will range between 55° and 72°C.[9] Optimal PCR annealing temperatures may be determined quickly using the RoboCycler Gradient 40 temperature cycler or RoboCycler Gradient 96 temperature cycler.

[f] For genomic targets 6–10kb and vector targets 10–15kb, use a 2 minute/kb extension time.

TROUBLESHOOTING

Observation	Possible cause	Solution(s)
No product or low yield	Extension times too short	Increase extension time to 2 minutes/kb of PCR target
	Annealing temperature too high	Lower the annealing temperature in 5°C increments
	Inappropriate buffer	Ensure that 10× cloned *Pfu* DNA polymerase reaction buffer is used
		Perform further buffer optimization if necessary using the Opti-Prime PCR optimization kit
	Order of addition of reaction components	Add *PfuTurbo* DNA polymerase last to the reaction mixture to minimize any potential primer degradation
	GC content or secondary structure too high	Use higher denaturing temperatures (94–98°C) (see also Reference 17)
		Use cosolvents such as DMSO in a 1–10% (v/v) final concentration or glycerol in a 5–20% (v/v) final concentration (see *Dimethylsulfoxide and Glycerol*)
	Primer concentration too low	Use the recommended primer concentrations between 0.1 and 0.5 μM (generally 100–250 ng for typical 18- to 25-mer oligonucleotide primers in a 100-μl reaction volume)
	Nonpriming or mispriming	Use high-quality primers
		Check the melting temperature, purity, GC content, and length of the primers
		Consider using the adjuncts from the Opti-Prime PCR optimization kit [e.g., use 1–2 U of Perfect Match PCR enhancer or a low concentration (1–5%) of formamide]

(*table continues on the next page*)

(*table continued from the previous page*)

Observation	Possible cause	Solution(s)
	Ionic strength of the reaction mixture too high	Remove extraneous salts from the PCR primers and DNA preparations
	Insufficient Mg^{2+} concentration	Increase the *total* Mg^{2+} concentration to 2 mM; take into account the concentrations of the dNTPs, primers, and EDTA
	Denaturation times too long	Denaturation times of 30–60 seconds at 94–95°C are usually sufficient while longer denaturation times may damage the DNA template; use the shortest denaturation time compatible with successful PCR on the thermal cycler
	Insufficient amounts of *PfuTurbo* DNA polymerase	Increase the amount of *PfuTurbo* DNA polymerase
	Poor template quality or template concentration too low	Use intact and highly purified templates at an adequate concentration (see *Primer–Template Purity and Concentration* and *Primer Design*)
	Inadequate heat exchange between the reaction tubes and the thermal cycler	Use thin-wall PCR tubes for Stratagene's thermal cyclers (i.e., the RoboCycler Gradient 96 temperature cycler, the RoboCycler 96 temperature cycler, the RoboCycler Gradient 40 temperature cycler, and the RoboCycler 40 temperature cycler). These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance
	Adjuncts or cosolvents required for optimal PCR	See the *Adjuncts and Cosolvents* section
Multiple bands	Primer annealing temperature too low	Increase the annealing temperature in 5°C increments
	Nonspecific primer–template annealing	Use Perfect Match PCR enhancer††† to improve PCR product specificity
Artifactual smears	Excessive amount of *PfuTurbo* DNA polymerase	Decrease the amount of *PfuTurbo* DNA polymerase
	Extension time too long	Reduce the extension time utilized

Preparation of Media and Reagents

10× Cloned *Pfu* DNA Polymerase Reaction Buffer

200 mM Tris-HCl (pH 8.8)
20 mM $MgSO_4$
100 mM KCl
100 mM $(NH_4)_2SO_4$
1% Triton® X-100
1 mg/ml nuclease-free BSA

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ENDNOTES

† Purchase of these products is accompanied by a license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with an Authorized Thermal Cycler. Stratagene's PCR products are sold under licensing arrangements with Roche Molecular Systems, Inc., F. Hoffmann-La Roche Ltd., and The Perkin-Elmer Corporation.

†† These products are optimized for use in the Polymerase Chain Reaction (PCR) process covered by patents owned by Hoffmann-La Roche. Purchase of these PCR-related products does not convey any rights under the PCR patents owned by Hoffmann-La Roche. A license to use the PCR process accompanies the purchase of certain reagents from Stratagene when used in conjunction with an Authorized Thermal Cycler.

††† Practice of the patented Polymerase Chain Reaction (PCR) process requires a license. Stratagene's thermal cycler is an Authorized Thermal Cycler. Its use with Authorized Reagents provides a limited PCR license in accordance with the label rights accompanying such reagents. It may also be used with PCR licenses available from The Perkin-Elmer Corporation.